Calcium Signaling Pathways

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Lysosome-Sarcoplasmic Reticulum Junctions: A Trigger Zone for Calcium Waves in Vascular Smooth Muscle
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We investigate a hypothesis for the genesis of nicotinic acid adenine dinucleotide phosphate (NAADP)-mediated Ca^{2+} waves in vascular smooth muscle. Agonist-stimulated waves of elevated cytoplasmic calcium concentration regulate blood vessel tone and vasomotion in vascular smooth muscle. In pulmonary artery smooth muscle cells, the calcium mobilizing messenger NAADP triggers bursts of Ca^{2+} release from lysosomes by activating the Two Pore Segment Channel subtype 2 (TPC2). These Ca^{2+} transients initiate a propagating wave by Ca^{2+}-induced Ca^{2+} release (CICR) from the SR via ryanodine receptors (RyRs). Results from deconvolution and confocal microscopy studies, including immunofluorescence, suggest that lysosome clusters may selectively couple to RyR subtype 3 (RyR3) in regions where lysosomes and proximal SR are separated by a narrow cleft possibly <100 nm and certainly beyond the resolution of light microscopy. These results naturally lead to the hypothesis that lysosome-SR (L-SR) junctions may form a cytoplasmic trigger zone for the observed Ca^{2+} bursts and subsequent cell-wide Ca^{2+} waves. The present study combines prior optical microscopy observations with a thorough ultrastructural characterization of the L-SR junctions as input data for a quantitative model of the junction to test the above hypothesis. With this model, we simulate the Ca^{2+} bursts that may be generated within L-SR junctions to determine whether or not these bursts give rise to a sufficient increase in junctional [Ca^{2+}] to breach the threshold for RyR3 activation by CICR and thus initiation of a propagating Ca^{2+} wave, and the degree to which this depends on the contribution to SR luminal Ca^{2+} load of concomitant SR Ca^{2+} uptake by sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA).

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P21-Activated Kinase (Pak1) is a Negative Regulator of ROS Generation in Ventricular Myocytes
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Pak1 a downstream target of Rac1 was implicated in the regulation of NADPH-oxidase (NOX2) activity. To determine the role of Pak1 in NOX2 dependent ROS generation isolated,DCF (10 μM) loaded, ventricular myocytes (VMs) were used and ROS measured during angiotensin II (AngII: 100 nM) stimulation. Decreased Pak1 activity in VMs treated with IPA-3 (10 μM) or in Pak1-/- VMs resulted in significantly increased ROS under basal conditions and during stimulation with AngII. The relevance of this increased ROS production was determined with an in vitro model of ischemia/reperfusion recording stimulation induced Ca transients in WT and Pak1-/- VMs with Fluo-4/AM. WT VMs exhibited an increase in diastolic [Ca]i (F/F0: 0.98 ± 0.04 to 1.28 ± 0.12, n=6, p< 0.05) during ischemia (15 min: 20 mM lactose, pH = 6.8, [K]o = 8 mM, gassed with 95% N2) but no arrhythmic activity. In Pak1-/- VMs the increase in diastolic Ca was significantly enhanced compared to WT VMs (F/F0: 1.95 ± 0.14, n=8 p<0.05) and Ca-induced arrhythmia were determined. The decay time constant of the caffeine induced calcium transient that reflects Na/Ca exchange (NCX) activity was significantly increased in Pak1-/- (WT: 3.28 ± 0.14 s, n=10; Pak1-/-: 2.32 ± 0.24 s, n=7 p< 0.05) while NCX protein level was unchanged. Treatment of Pak1-/- VMs with the NCX reverse-mode blocker KBR-7943 (5 μM) (F/F0: 1.13 ± 0.06, n=4 p<0.05), the ROS scavenger TEMPOL (F/F0: 1.31 ± 0.04, n=4 p<0.05) or the NOX2 inhibitor apocynin (200 μM) (F/F0: 1.19 ± 0.05, n=4 p<0.05) attenuated ischemia induced Ca overload and arrhythmia in Pak1-/-. Our results suggest that Pak1 negatively regulates ROS production through NOX2 and that a NOX2/ROS dependent increase in NCX activity is a source for arrhythmia under pathophysiological conditions.

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Activation of β3-Adrenoceptors Induces Increase in Intracellular Free Zinc Ion via No Signaling Pathway in Hyperglycemic Cardiomyocytes
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Hyperglycemia is a major etiological factor causing cardiovascular dysfunction. Changes in intracellular ionic homeostasis via a series of biochemical changes initiated by hyperglycemia directly affect cellular function resulting abnormal cardiac remodeling and development of diabetic complications. Since hyperglycemia regulates free cytosolic Zn2+ ([Zn2+]i) and attenuates protein S-nitrosylation via NO-cGMP-PKG, which induces depressed cardiac contractility, in part contribution of activated β3-adrenergic receptor (β3-AR). We postulated a possible cross-talk between hyperglycemia, activation of β3-AR and NO availability, [Zn2+]i increase and contractile dysfunction. [Zn2+]i levels were determined by imaging freshly isolated adult rat cardiomyocytes from either normoglycemic or hyperglycemic rat loaded with FluoZin-3. We investigated the distribution of β-ARs subtypes by measuring the expression/protein levels of β1-, β2-, β3-AR as well as their left ventricular agonist responsiveness using isolated perfused hearts from diabetic rats. We found that hyperglycemia influenced both function and the mRNA/protein levels of selective β1- and β3-AR although the total β-AR did not change. In vitro experiments showed that β3-AR agonist (BRL-37,344) induced significant increase in [Zn2+]i while this effect was prevented with L-NAME exposure in normoglycemic cardiomyocytes. Our data demonstrated that either chronic or acute hyperglycemia initiates a series of interconnected biochemical changes in cardiomyocytes including a cross-talk between activation of β3-AR and NO signaling pathway including increased [Zn2+]i and then this cross-talk meadiates abnormal cardiac remodeling and the development of diabetic cardiomyopathy. Therefore, it can be proposed a pathway that a dynamic association of intracellular free Zn2+ with sulfur in protein cysteine clusters under hyperglycemia, from which the metal is released by NO and other thiol oxidant species is one of the mechanisms which plays important role in the Zn2+ contribution to maintaining cellular redox balance. (Supported by TUBITAK-SBAG-109S267 & 111S042)

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The Involvement of NAADP and Two-Pore Ca^{2+} Channels in the Cardiac Beta-Adrenergic Response
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NAADP is a potent endogenous Ca^{2+}-mobilising agent with actions in many cell types, including cardiac myocytes[1,2]. Our previous work suggests that NAADP causes Ca^{2+} release from an acidic endolysosomal store, leading to additional Ca^{2+} uptake by the SR[1,2]. Endolysosomal Ca^{2+} release is thought to occur via a two-pore Ca^{2+} channel (TPC), which exists in several subtypes including TPC1 and TPC2[3,4]. The aim of this study was to investigate whether Ca^{2+} mobilised by NAADP is important in cardiac beta-adrenergic signalling. In guinea pig ventricular myocytes, photorelease of NAADP elicited an increase in the amplitude of the Ca^{2+} transient (42 ± 11% from control; n=6, P<0.05). Isoprenaline increased the amplitude of the Ca^{2+} transient by 148 ± 6% (n=10, P < 0.05); this response was reduced to 102 ± 12% in the presence of bafilomycin (to disrupt the endolysosomal Ca^{2+} store; n=6, P<0.05) and to 106 ± 6% in the presence of NED-19, an inhibitor of the NAADP pathway (n=6, P<0.05).
In ventricular myocytes from transgenic mice lacking TPC1 and 2 proteins (TPCDKO), the increase in amplitude of contraction in response to isoprenaline was reduced from 243 ± 31% in wild type (WT) to 140 ± 24% in TPCDKO (both n=8, P<0.05). In Langendorff-perfused hearts lacking TPC2 proteins (TPC2KO), the isoprenaline-induced increase in contractile force was reduced from 92 ± 4% in WT (n=5) to 63 ± 9% in TPC2KO hearts (n=7, P<0.05).
These data support the hypothesis that NAADP-induced Ca^{2+} mobilisation contributes to the cardiac beta-adrenergic response. It appears that TPC proteins are involved in this mechanism, presumably as a consequence of increased synthesis of NAADP.
1. Collins *et al*., (2011) *Cell Calcium* 50: 449-458
2. Macgregor *et al.,* (2007) *J Biol Chem* 282: 15302-15311
3. Calcraft *et al.,* (2009) *Nature* 459: 596-60
4. Elson *et al*., (2010) *Biophys J* 98: 100a

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Selective Activation of Epac Increases the Frequency of Submembrane Calcium Sparks in Mesenteric Smooth Muscle Cells
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The relaxation of vascular smooth muscle, which increases blood vessel diameter, is often mediated through vasodilator-induced elevations of intracellular cyclic AMP (cAMP) [1]. The vasculature expresses three distinct cAMP effectors: cAMP-dependent protein kinase (PKA), cyclic nucleotide-gated (CNG) ion channels and the more recently discovered exchange proteins directly activated by cAMP (Epacs) [2]. The mechanisms by which cAMP induces vasorelaxation are thus complex and diverse. Here we investigate the hypothesis that Epac activation increases the frequency of subsurface Ca^{2+} sparks within rat mesenteric smooth muscle cells, activating large-conductance Ca^{2+}-activated